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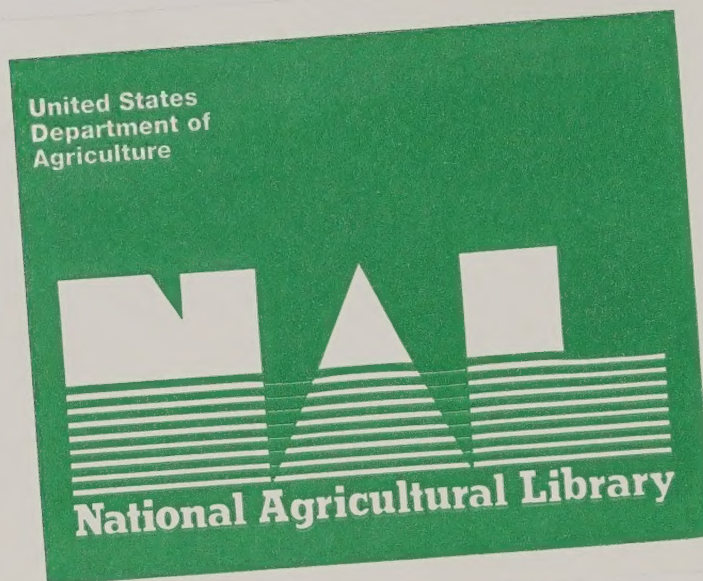January 2004

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# SOSS

## Phase II: Scrapie: Ovine Slaughter Surveillance Study 2002-2003

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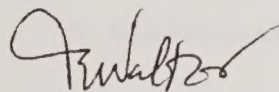www.aphis.usda.gov/vs/ceah/cahm

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# Acknowledgments

# Table of Contents

# Introduction

The purpose of the SOSS study was to estimate the regional and national prevalence of scrapie in mature cull sheep in the United States.

Phase I of SOSS was conducted from February 2001 through March 2002 and included refinement of the study design and sample collection training. The purpose of Phase I was to develop and modify the sample collection and testing processes, without emphasizing statistical results (see appendix III).

SOSS Phase II is similar to Phase I in that similar sample collection procedures and testing were used, along with a representative sample allocation. Beginning April 1, 2002, and continuing through March 31, 2003, Phase II included the collection of tissue samples from 12,508 sheep from 22 slaughter plants and 1 large livestock market.

Prior to the SOSS study, the estimated prevalence of scrapie in the United States was 0.07 percent (based on unpublished data from the NAHMS Sheep '96 study). However, the Sheep '96 estimate was based on a mail-in survey of producers who reported the presence of suspected or confirmed cases of scrapie in their flock over a period of 5 years, including lambs and mature sheep. The flock estimate was then expanded based on flock size to generate the animal-level prevalence estimate. The results of the SOSS study cannot be directly compared to the Sheep '96 prevalence estimate because of differences in study design, reference population, and data collection methods.

**Terms Used in this Report**

**Age:** Determined by examining the teeth (number of permanent incisors) in carcass heads presented for sampling (see appendix V).

**Face color:** A phenotypic characteristic of sheep associated with breed.
  **White:** Wool and/or hair on face are primarily white in color (e.g., Rambouillet and Dorset breeds).
  **Black:** Wool and/or hair on face are primarily black in color (e.g., Suffolk and Hampshire breeds).
  **Mottled:** Some wool and/or hair on the face is darker and form spots, freckles, or blotches that create a mottled appearance (usually associated with crossbreds or composites).

**Fiscal year, 2002 (FY02):** Time period from October 1, 2001, through September 30, 2002.

**Genotypes:** A codon is defined as a set of three nucleotides that encode for a specific amino acid. Three codons that encode for amino acids at postions 136, 154, and 171 in the PrP protein have been associated with scrapie susceptibility in sheep in the United States. However, codon 171 is thought to be the major determinant of scrapie susceptibility in the United States.

PrP genotypes are reported as the diploid PrP genotype at codons, 136, 154, and 171, such as QQ at codon 171. Amino acid single-letter abbreviations are shown below:

  A: alanine
  H: histidine
  K: lysine
  Q: glutamine
  R: arginine
  V: valine

**Mature sheep:** A sheep 1 year of age or older.

**No test:** Samples could not be genotyped because of insufficient DNA or other reasons.

**Obex:** A specific anatomical location in the brainstem between the cerebellum and the medulla oblongata where a v-shaped portion of the fourth ventricle of the brain is formed. This location is a critical area for sampling because of the accumulation of the scrapie prion protein in this region.

**Examples of a 95% Confidence Interval**



Standard Errors

**Prevalence estimates:** Estimates in this report were statistically weighted to reflect the population from which the sample was selected and are provided with a measure of precision called the **standard error**. A 95-percent confidence interval can be created with bounds equal to the estimate, plus or minus two standard errors. If the only error is sampling error, the confidence intervals created in this manner will contain the true population mean 95 out of 100 times. For example, an estimate of 7.5 with a standard error of 1.0 results in limits of 5.5 to 9.5 (two-times the standard error above and below the estimate). The second estimate of 3.4 shows a standard error of 0.3 and results in limits of 2.8 and 4.0. Alternatively, the 90-percent confidence interval would be created by multiplying the standard error by 1.65 instead of 2. In general, when comparing point estimates between categories, estimates with confidence levels that overlap are not considered different. Most estimates in this report are rounded to the nearest hundreth. If rounded to 0, the standard error was reported. If there were no reports of the event, no standard error was reported.

**Regions/States:**

> **West:** California, Oregon, and Washington
>
> **Mountain:** Arizona, Colorado, Idaho, Montana, Nevada, New Mexico, Oklahoma, Texas, Utah, Wyoming
>
> **Central:** Iowa, Kansas, Minnesota, Missouri, Nebraska, North Dakota, South Dakota
>
> **East:** Alabama, Arkansas, Connecticut, Delaware, Florida, Georgia, Illinois, Indiana, Kentucky, Louisiana, Maine, Maryland, Massachusetts, Michigan, Mississippi, New Hampshire, New Jersey, New York, North Carolina, Ohio, Pennsylvania, Rhode Island, South Carolina, Tennessee, Vermont, Virginia, West Virginia, Wisconsin
>
> **Multiregion:** Unable to trace animals to a specific region (e.g., a group of animals gathered from numerous States in different regions and sold at a livestock market).
>
> **National:** All regions combined, including multiregion.

**States/Regions**



West
Mountain
Central
East
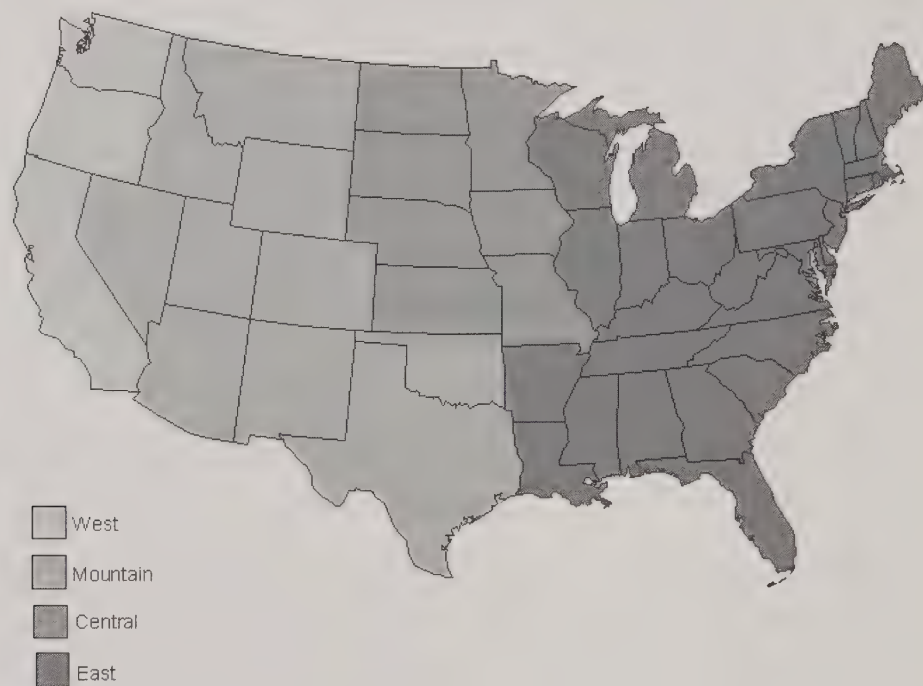
**Sample profile:** Information that describes characteristics of the sample from which SOSS data were collected (see appendix I).

**Weighted estimates:** Sample estimates expanded to statistically represent the population being considered (see Section II: Methodology, D. Population Inferences).

# Section I: Prevalence Estimates

**A. Weighted Test Results**

**1. Overall prevalence**

Of the 12,508 mature sheep sampled, valid (at least one testable tissue) test results were obtained from 12,491 (99.9 percent). A positive result was recorded for any animal that tested positive by immunohistochemistry (IHC) **on one or more of the tissues sampled**. The overall weighted national prevalence of scrapie in mature sheep is 0.20 percent. Estimates could not be made in the West region due to the low number of samples obtained. However, national estimates include samples collected in the West region.

a. Percentage of sheep that tested positive for scrapie, by region*:

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Percent Sheep** | | | | | | | | | |
| **Region** | | | | | | | | | |
| **Mountain** | | **Central** | | **East** | | **Multiregion** | | **National** | |
| **Pct.** | **Std. Error** | **Pct.** | **Std. Error** | **Pct.** | **Std. Error** | **Pct.** | **Std. Error** | **Pct.** | **Std. Error** |
| 0.14 | (0.06) | 0.21 | (0.10) | 0.52 | (0.15) | 0.13 | (0.07) | 0.20 | (0.04) |

*Because of the low number of samples obtained in the West region, results for the West region are included in the National estimates but are not listed individually.

**Percent of Sheep that Tested Positive for Scrapie, by Region***
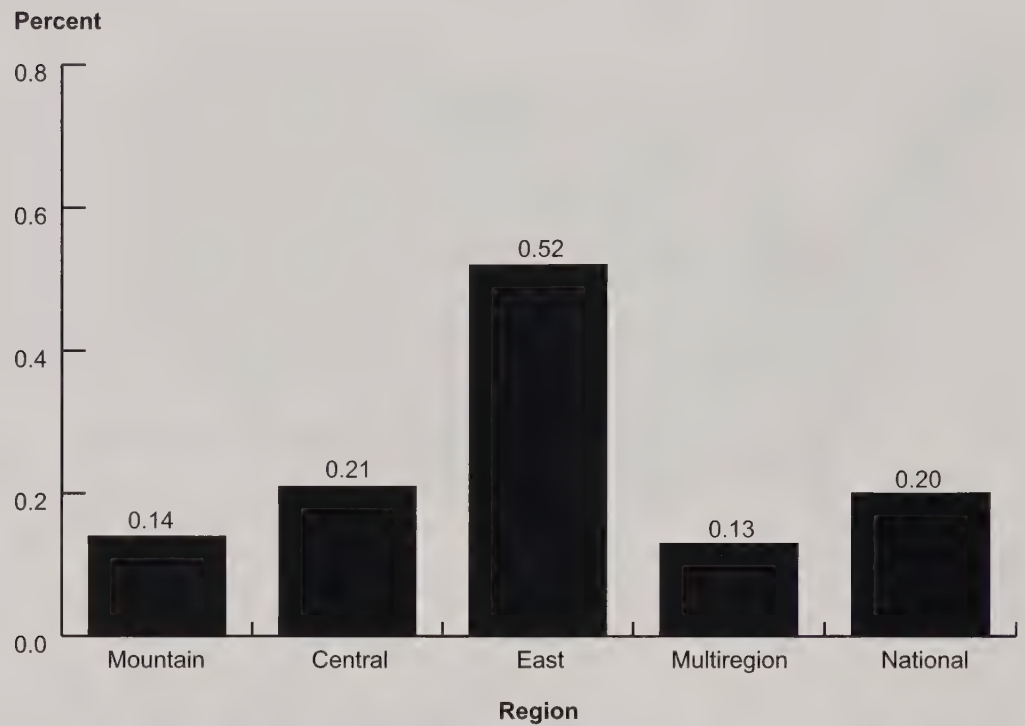
**Percent**



*Because of the low number of samples obtained in the West region, results for the West region are included in the National estimates but are not listed individually.

## 2. Prevalence by tissue type

Three tissue types (obex, tonsil, and retropharyngeal lymph node) were collected from each sheep head for IHC testing. As expected, each tissue type differed slightly in the number tested as well as the number of positive results; however, the prevalence was similar for the three tissue types.

a. Percentage of sheep that tested positive for scrapie, by tissue type:

| Tissue Type | Percent | Standard Error |
|---|---|---|
| Obex | 0.18 | (0.04) |
| Tonsil | 0.19 | (0.04) |
| Retropharyngeal lymph node | 0.20 | (0.04) |

**Percent of Sheep That Tested Positive for Scrapie, by Tissue Type**
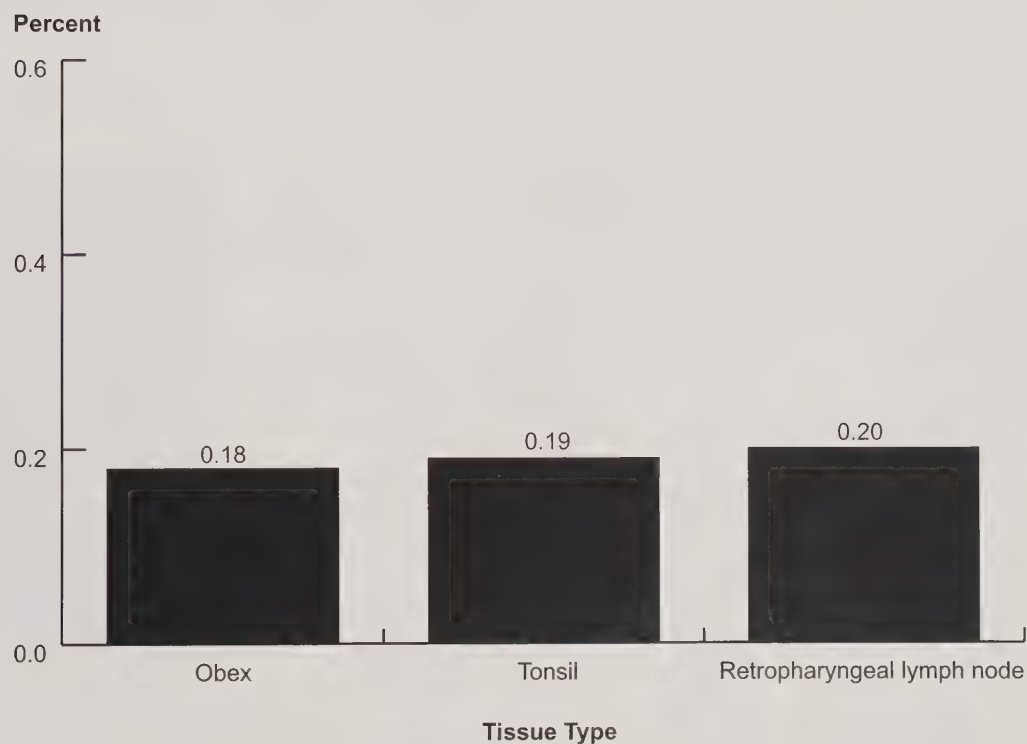
**Percent**

### 3. Prevalence by face color

Scrapie prevalence (one or more tissue samples tested positive) was highest in black-faced sheep (0.84 percent). White-faced sheep were far less likely to test positive for scrapie (less than 0.01 percent). Some animals were presented for sample collection with the skin removed. Therefore, face color could not be determined on these animals and they were not included in these estimates.

a. Percentage of sheep that tested positive for scrapie, by face color.

| Face Color | Percent Sheep | Standard Error |
|---|---|---|
| White | 0.00 | (0.00) |
| Black | 0.84 | (0.19) |
| Mottled | 0.12 | (0.07) |

**Percent of Sheep that Tested Positive for Scrapie, by Face Color**
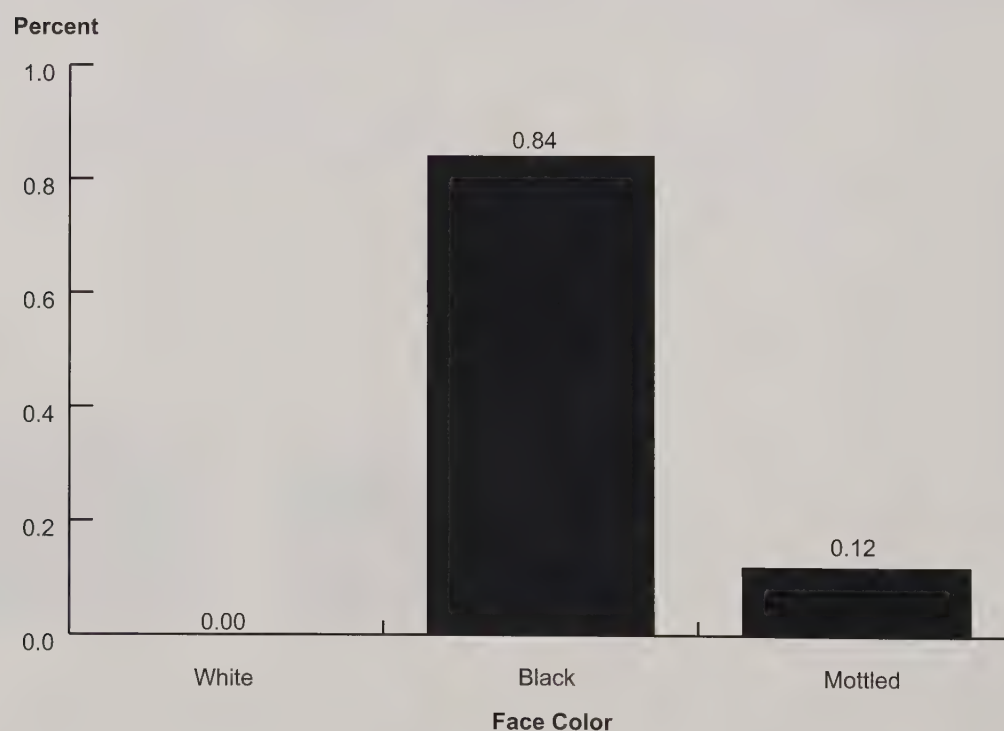
**Percent**

## 4. Prevalence by age

Age was determined based on the number of visible permanent incisors (see appendix V). Four-year-old sheep tested positive (one or more tissue samples tested positive) most frequently (0.49 percent of sheep tested). Scrapie prevalence increased with age until the animals reached 4 years old, then decreased.

a. Percentage of sheep that tested positive for scrapie (national and black-faced sheep), by age:

| | Percent Sheep | | | |
| | National | | Black-faced | |
| Age | Percent | Standard Error | Percent | Standard Error |
|---|---|---|---|---|
| 1 and 2 years | 0.11 | (0.07) | 0.00 | (--) |
| 3 years | 0.21 | (0.11) | 0.54 | (0.32) |
| 4 years | 0.49 | (0.16) | 1.77 | (0.60) |
| 5 years or more | 0.13 | (0.04) | 0.69 | (0.24) |

**Percent of Sheep that Tested Positive for Scrapie (National and Black-faced Sheep), by Age**

Percent
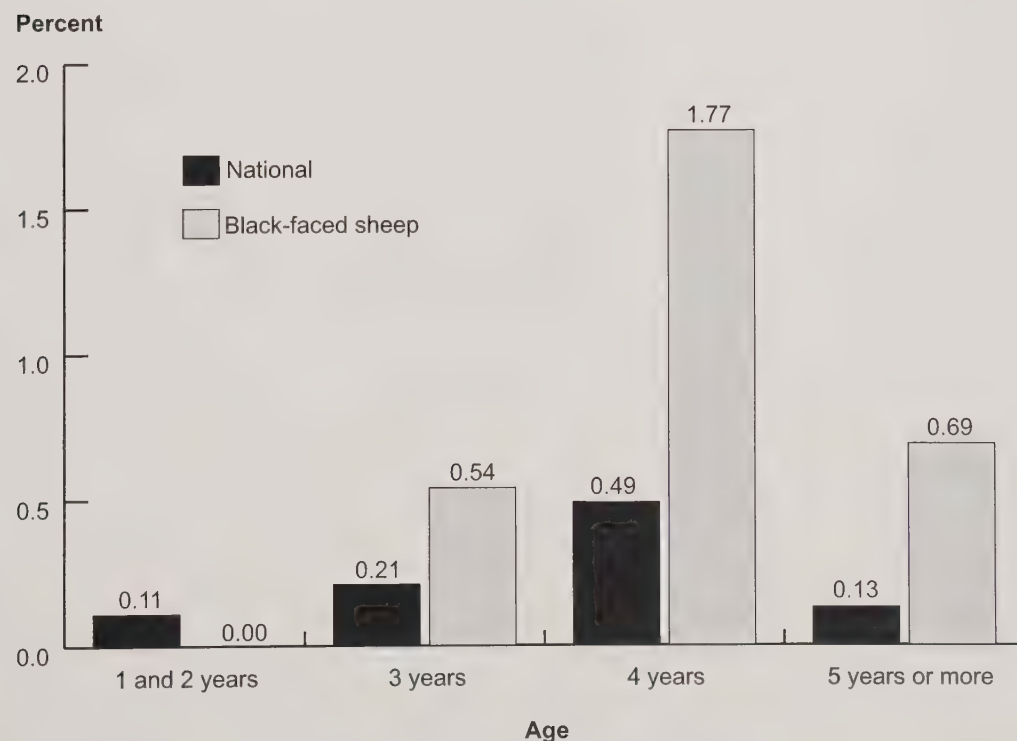
### 5. Logistic regression model

Black-faced sheep were significantly more likely to test positive for scrapie than white- or mottled-faced sheep (OR = 38, $p < 0.001$), even when adjusting for age and region. Age was marginally significant in association with scrapie ($p = 0.06$), with 4-year-old sheep more likely to test positive for scrapie than sheep younger or older than 4 years. Although the East region had the highest scrapie prevalence, the difference was not significant after adjusting for face color and age.

a. Association of face color, age, and region with scrapie:

| Factor | Odds Ratio | 95-Percent Confidence Interval | P-value |
|---|---|---|---|
| Black Face: | | | |
| Yes | 38.4 | 10.5–139.9 | Less |
| No | 1.0 | | than 0.001 |
| Age: | | | |
| Less than 4 years | 0.6 | 0.2–1.7 | |
| 4 years | 2.1 | 0.7–6.0 | 0.06 |
| More than 4 years | 1.0 | | |
| East Region: | | | |
| Yes | 1.6 | 0.6–4.3 | |
| No | 1.0 | | 0.36 |

**Note**: The odds (OR) ratio is a measure of the risk of an outcome (e.g., scrapie) for one group (e.g., blackface) compared to a reference group (e.g., white or mottled face). The 95-percent confidence interval indicates the precision of the OR estimate (e.g., the OR for face color could be anywhere from 10.5 to 139.9). The p-value measures the likelihood that the association is due to chance (e.g., face color vs. scrapie; there is less than a 0.1 percent probability this association is due to chance alone).

## B. Unweighted Genetic Results

Two laboratories were sent cerebellum samples for genetic testing. The first laboratory reported results for codon 171 only; the second laboratory reported results for codons 136, 154, and 171. In addition, the second laboratory tested for the K and H alleles at codon 171. The significance of these alleles to scrapie susceptibility is still being researched.

### 1. Distribution of sampling

The first laboratory conducted genetic testing on 1,245 randomly selected cerebellum samples. The RR genotype accounted for 15.74 percent of sheep tested at the first laboratory. In addition, all sheep that tested positive for scrapie were submitted for genetic testing.

a. Genotype distribution for tested cerebellum samples (first laboratory), by genotype at codon 171:

| Genotype (Codon 171) | Number Cerebellum Samples | Percent |
| --- | --- | --- |
| QQ | 506 | 40.64 |
| QR | 543 | 43.62 |
| RR | 196 | 15.74 |
| Total | 1,245 | 100.00 |

The second laboratory tested 1,784 cerebellum samples, including 79 "no test" samples from the first laboratory. Also, as a quality control measure, 124 samples from the first laboratory were randomly selected and tested at the second laboratory. In addition, all positive samples were retested at the second laboratory, if tissues were available (see table c, next page).

b. Genotype distribution for all tested cerebellum samples (first and second laboratories), by genotype at codon 171:

| Genotype (Codon 171) | Number Cerebellum Samples | Percent |
|---|---|---|
| QQ | 1,138 | 39.31 |
| QR | 1,291 | 44.60 |
| RR | 454 | 15.68 |
| H* | 9 | 0.31 |
| K** | 3 | 0.10 |
| Total | 2,895*** | 100.00 |

*Includes: HH, QH, RH (second lab)
** Includes: KQ, KR (second lab)
*** Adding the total samples tested at the first laboratory (table a) to the total samples tested at the second laboratory (table c) does not equal the total in table b. This discrepancy is due to duplicate testing and "no test" results.

c. Genotype distribution for tested cerebellum samples (second laboratory), by genotype at codons 136, 154, and 171:

| Genotype (Codons 136, 154, 171) | Number Cerebellum Samples | Percent |
|---|---|---|
| **/RR/QQ | 1 | 0.06 |
| AA/**/QQ | 4 | 0.22 |
| AA/**/QR | 10 | 0.56 |
| AA/**/RR | 2 | 0.11 |
| AA/HH/QQ | 5 | 0.28 |
| AA/HR/QQ | 55 | 3.08 |
| AA/HR/QR | 30 | 1.68 |
| AA/HR/RR | 2 | 0.11 |
| AA/RR/** | 2 | 0.11 |
| AA/RR/HH | 1 | 0.06 |
| AA/RR/KQ | 2 | 0.11 |
| AA/RR/KR | 1 | 0.06 |
| AA/RR/QH | 4 | 0.22 |
| AA/RR/QQ | 531 | 29.77 |
| AA/RR/RH | 4 | 0.22 |
| AA/RR/QR | 695 | 38.96 |
| AA/RR/RR | 265 | 14.86 |
| VA/**/QQ | 1 | 0.06 |
| VA/**/QR | 2 | 0.11 |
| VA/HH/QQ | 1 | 0.06 |
| VA/HR/QQ | 9 | 0.50 |
| VA/RR/QQ | 86 | 4.82 |
| VA/RR/QR | 63 | 3.53 |
| VV/RR/QQ | 8 | 0.45 |
| Total | 1,784 | 100.00 |

**No test

d. Genotype distribution for tested cerebellum samples (second laboratory), by genotype at codon 136:

| Genotype (Codon 136) | Number Cerebellum Samples | Percent |
|---|---|---|
| AA | 1,613 | 90.47 |
| VA | 162 | 9.08 |
| VV | 8 | 0.45 |
| Total | 1,783 | 100.00 |

e. Genotype distribution for tested cerebellum samples (second laboratory), by genotype at codons 136 and 171:

| Genotype (Codons 136, 171) | Number Cerebellum Samples | Percent |
|---|---|---|
| AA/HH | 1 | 0.06 |
| AA/KQ | 2 | 0.11 |
| AA/KR | 1 | 0.06 |
| AA/QH | 4 | 0.22 |
| AA/QQ | 595 | 33.35 |
| AA/QR | 735 | 41.20 |
| AA/RH | 4 | 0.22 |
| AA/RR | 269 | 15.08 |
| VA/QQ | 97 | 5.44 |
| VA/QR | 65 | 3.64 |
| VV/QQ | 8 | 0.45 |
| AA/** | 2 | 0.11 |
| **/QQ | 1 | 0.06 |
| Total | 1,784 | 100.00 |

**No test

Tissue samples from the 33 sheep that tested positive for scrapie were submitted for genetic testing. All 33 samples were of the QQ genotype at codon 171. This genotype has been characterized as the least resistant to scrapie.

f. Genotype distribution for postive cerebellum samples (second laboratory), by genotype at codons 136, 154, and 171:

| Genotype (Codons 136, 154, 171) | Number Cerebellum Samples | Percent |
|---|---|---|
| AA/RR/QQ | 30 | 90.91 |
| **/RR/QQ | 1 | 3.03 |
| VA/RR/QQ | 2 | 6.06 |
| Total | 33 | 100.00 |

**No test

# Section II: Methodology

**A. Background**

**1. Mature sheep slaughter and marketing**

Federally-inspected slaughter for 2002 accounted for 94.1 percent of the total commercial slaughter. There were 148,000 mature sheep slaughtered in Federally-inspected plants in 2002. Slaughter numbers for tables a and b were taken from the Livestock Slaughter Annual Summary published by NASS.

a. Sheep and lamb slaughter in the United States (1,000 head):

| | **Sheep and Lamb Slaughter[1] (1,000 Head)** | | | | | |
|---|---|---|---|---|---|---|
| | **Commercial Slaughter** | | | | **Farm Slaughter** | **Total Slaughter[2]** |
| | **Federally Inspected** | | | **Other[3]** | | |
| **Year** | **Mature Sheep** | **Lambs** | **Total** | **Sheep and Lambs** | **Sheep and Lambs** | **Sheep and Lambs** |
| 1999 | 188 | 3,368 | 3,556 | 145 | 65 | 3,766 |
| 2000 | 167 | 3,141 | 3,308 | 152 | 67 | 3,527 |
| 2001 | 144 | 2,921 | 3,065 | 157 | 68 | 3,290 |
| 2002 | 148 | 2,944 | 3,092 | 194 | 65 | 3,351 |

[1]NASS Livestock Slaughter Annual Summary
[2]Total commercial slaughter plus farm slaughter
[3]State and other nonfederally inspected commercial slaughter

The number of mature sheep slaughtered varies only slightly from month to month. For 2002, mature sheep slaughter ranged from a low of 10,000 head in January to a high of 14,000 head in May and October.

b. Monthly distribution of Federally-inspected mature sheep slaughter in the United States (1,000 head):

| | Mature Sheep Slaughter[1] (1,000 Head) | | | |
|---|---|---|---|---|
| Year | 1999 | 2000 | 2001 | 2002 |
| January | 13 | 13 | 12 | 10 |
| February | 13 | 13 | 10 | 11 |
| March | 17 | 15 | 13 | 11 |
| April | 17 | 13 | 12 | 12 |
| May | 15 | 16 | 13 | 14 |
| June | 17 | 15 | 12 | 12 |
| July | 15 | 13 | 12 | 13 |
| August | 16 | 15 | 12 | 12 |
| September | 17 | 14 | 10 | 13 |
| October | 15 | 14 | 13 | 14 |
| November | 16 | 13 | 12 | 13 |
| December | 17 | 13 | 13 | 13 |
| Total | 188 | 167 | 144 | 148 |

[1]Nass Livestock Slaughter Annual Summary

## 2. Mature sheep marketed to Mexico

The San Angelo, Texas market sold approximately 244,000 mature sheep in 2002 (estimated based on a summary of weekly auction reports), of which approximately 201,000 (82.5 percent) were exported to Mexico. This market was selected for the SOSS study because large numbers of mature sheep are shipped to Mexico through this market and thus are not represented in U.S. slaughter plants. The following table shows: a recent history of the number of sheep and lambs exported to Mexico (Foreign Agricultural Service (FAS)); the Texas live-market volume; the estimated number of mature sheep sold through the market (60 to 65 percent of the market volume); and that a high percentage (80 to 85 percent) of the mature sheep sold through the market go to Mexico (USDA-Texas Department of Agriculture Market News estimate).

a. Annual sheep exports to Mexico (FAS) and sheep sold at the Texas live market (USDA-Texas Department of Agriculture Market News estimate):

| | Live Sheep Exported to Mexico[1] | Texas Live Market[2] | | | | |
| | | Total Sheep and Lambs | Mature Sheep | | Mature Sheep to Mexico | |
| Year | No. (1,000 Head) | No. (1,000 Head) | No. (1,000 Head) | Est. Pct. of Total | No. (1,000 Head) | Est. Pct. of Mature |
|---|---|---|---|---|---|---|
| 1999 | 435 | 404 | 253 | 62.6 | 209 | 82.5 |
| 2000 | 378 | 448 | 269 | 60.0 | 222 | 82.5 |
| 2001 | 383 | 337 | 211 | 62.5 | 174 | 82.5 |
| 2002 | 403 | 390 | 244 | 62.5 | 201 | 82.5 |

[1]Foreign Agricultural Service (FAS)
[2]USDA—Texas Department of Agriculture Market News

### 3. Projected number of sheep culled

a. In the United States, the number of mature sheep culled in a single year varies and is difficult to measure directly. Four different methods were used to project or estimate the number of head culled in 2002:

| Method | Projected Number of Sheep Culled in 2002 |
|---|---|
| 1.  FSIS FY02 mature sheep slaughter | 144,201 |
|     +Export of live animals to Mexico | 403,000 |
|     =Cull indicator or total culled | 547,201 |
| 2.  NASS annual mature slaughter | 148,000 |
|     +Export of live animals to Mexico | 403,000 |
|     =Cull indicator or total culled | 551,000 |
| 3.  Ewes* (Jan. 1 end-of-year NASS inventory) | 3,789,000 |
|     Rams* (Jan. 1 end-of-year NASS inventory) | 191,500 |
|     Ewe cull rate @ 18.3 percent (NAHMS) | 693,387 |
|     + Ram cull rate @ 23.8 percent (NAHMS) | 45,577 |
|     =Ewes and rams culled | 738,964 |
|     x Mature culled @ 90 percent (estimate) | 665,068 |
| 4.  Replacement lambs (Jan. 1 end-of-year NASS inventory) | 701,500 |
|     - Sheep deaths (NASS) | (263,900) |
|     =Cull indicator or total culled | 437,600 |

*Ewes/Rams 1 year or older

Methods 1 and 2 use similar calculations but different slaughter components. Method 1 uses FY02 data and sums the mature sheep slaughter in Federally-inspected plants (144,201 head) and the number of live sheep exported to Mexico (403,000 head) for a total of 547,201 head culled. Method 2 uses NASS annual slaughter data for mature sheep and yields a total of 551,000 head culled. The results from these two slaughter indicators are similar. The results assume that: most culled sheep that remain in the United States are slaughtered in Federally-inspected facilities; most live sheep exported to Mexico are cull sheep; and that there is not a substantial number of cull sheep exported to other countries.

The third method applies the overall culling rate. Data from the NAHMS Sheep 2001 study indicated that 18.3 percent of ewes and 23.8 percent of rams were culled. Further, it is roughly estimated that 90 percent of culled sheep are mature sheep, resulting in an estimate for 2002 of 665,068 head. However, the 2002 estimate may be high since sheep culled from one producer could have ended up in another producer's inventory and been counted twice.

The fourth method uses the NASS end-of-year estimate for the number of replacement lambs. NASS estimated the number of replacement lambs for January 1, 2003, to be 701,500 head. These lambs not only replaced the cull breeding stock but also replaced any mature sheep death loss that occurred during the year. NASS provides separate estimates of death loss for sheep and for lambs. The resulting indicator for number culled in 2002 is 437,600 head.

For all four methods, these approaches yield estimates for number of sheep culled in 2002 at 547,201, 551,000, 665,068, and 437,600 head, respectively. The two slaughter approaches yield similar results. The two inventory approaches vary widely on each side of the slaughter approaches. For SOSS purposes, a rounded "average" of 550,000 head was chosen to represent the cull sheep population.

If the U.S. slaughter is estimated at 148,000 (NASS), then the remaining 403,000 are exported (primarily to Mexico). In 2002 an estimated 244,000 cull sheep moved through the market at San Angelo, Texas (USDA-Texas Department of Agriculture Market News). Of these 244,000 animals, approximately 80 to 85 percent (201,000) went to Mexico and the rest remained in the United States. Thus, the San Angelo market represents about 50 percent (201,000/403,000) of the mature sheep export to Mexico.

**B. Sampling**

**1. Total sample size**

A minimum sample size requirement of 11,000 was calculated based on the previous prevalence estimate of 0.07 percent (+/- .05 percent, with 95-percent confidence). The actual sampling plan exceeded this estimate in order to ensure an adequate sample size.

A total of 12,508 animals were sampled between April 1, 2002, and March 31, 2003. Obex, tonsil, and lymph-node tissues from each sheep were tested using the immunohistochemistry (IHC) technique at NVSL and its approved laboratories (see appendix IV). A positive case was defined as having a positive test result on any tissue. Approximately one-fourth of the cerebellum samples were submitted for genetic testing. In addition, all sheep that tested positive for scrapie were submitted for genetic testing.

**2. Plant selection and sampling allocation**

Slaughter plants were selected based on historical slaughter of mature sheep at Federally-inspected slaughter facilities (FSIS FY2001 data). The 21 highest volume FSIS-inspected plants were selected. In addition, a high-volume State-inspected plant was selected. Plants were grouped into strata based on volume. Each stratum was allocated samples based on historic volume for the stratum. Each plant stratum was then allocated a weekly sampling goal for a total of 15,050 samples allocated for the 50-week collection period. Collections from smaller plants were often taken biweekly or monthly due to the fluctuations in mature sheep slaughter times at these facilities.

a. Number of samples allocated:

| Stratum | No. Plants | Samples Per Week Per Stratum | Samples Per Week Per Plant | 50-Week Total Per Stratum | 50-Week Total Per Plant | Total Samples Collected |
|---------|-----------|--------------|------------|--------------|------------|-------------------|
| 1 (mkt.) | 1 | 150 | 150 | 7,500 | 7,500 | 6,969 |
| 2 | 5 | 75 | 15 | 3,750 | 750 | 3,052 |
| 3 | 5 | 40 | 8 | 2,000 | 400 | 1,319 |
| 4 | 12 | 36 | 3 | 1,800 | 150 | 1,168 |
| Total | 23 | 301 | | 15,050 | | 12,508 |

### 3. Animal selection

Sample collectors were instructed to collect samples using systematic sampling. Sheep were selected based on the number of animals needed to meet the weekly plant allocation.

### 4. Laboratory allocations

Samples were submitted to NVSL for testing or for distribution to approved laboratories. Laboratory distribution was dependent on the number of laboratories with available testing capacity. Efforts were made to evenly distribute samples from each plant to all participating laboratories.

For quality control, all positive samples and one-eighth of all negative samples were retested by NVSL to confirm the results.

### 5. Genetic testing

One in four cerebellum samples was randomly selected for genetic testing using a random numbers table. In addition, all positive cerebellum samples were submitted for genetic testing. One laboratory provided codon 171 results. A second laboratory provided results at codons 136, 154, and 171.

## C. Region of Origin

Sheep were traced to State of origin based on ear tags and/or other information obtained by the collector at the plant or market. For analysis purposes, samples identified to individual States were assigned to one of four defined regions. Sometimes only a listing of multiple States could be obtained for a group of sheep (e.g., market animals accumulated across numerous states). These samples were assigned to the Multiregion category if the States they came from were not all in the same region. In cases where a trace State was not identified by the collector (n = 2,020), a region was assigned based on their official identification information. The 2001 NAHMS Sheep study showed that at least 95 percent of cull sheep movement was within the region of origin.

## D. Population Inferences

The study included 22 major mature sheep slaughter plants throughout the United States (21 FSIS inspected, 1 State plant), and one large livestock market in Texas. Inferences cover the population of mature sheep from these sources. The 21 FSIS plants represented approximately two-thirds of the total FSIS mature sheep slaughtered during the study period. The livestock market represented approximately one-half of the live sheep exported to Mexico. All sample data were statistically weighted to reflect the population from which the sample was selected. The number of samples collected from each plant on a specific day was statistically weighted to represent the volume of mature sheep slaughtered (sold) through each plant (market) that specific day. This weight was adjusted for the total volume of mature sheep through the plant (market) from April 2002 through March 2003. Results of genotype testing were unweighted because of the small sample size. Overall, the samples collected from the 22 plants and the livestock market represented 299,000 sheep (54 percent of the cull sheep population, estimated at 550,000 head).

## E. Data Validation and Analysis

Data were double-entered into SAS data sets. Data were validated using double entry comparison (SAS Proc Compare). Additional data validation was performed by APHIS/VS/CEAH staff after all data were entered. Final analysis was accomplished using SUDAAN software.

# Appendix I: Sample Profile

a. Number of samples submitted, by face color and by region:

| | | | Samples Submited | | | |
| | | | Region | | | |
| Face Color | West | Mountain | Central | East | Multiregion | Total |
|---|---|---|---|---|---|---|
| Black | 100 | 535 | 680 | 1,023 | 453 | 2,791 |
| White | 493 | 2,997 | 1,993 | 1,283 | 1,472 | 8,238 |
| Mottled | 71 | 305 | 413 | 404 | 194 | 1,387 |
| Unknown | 6 | 32 | 4 | 42 | 8 | 92 |
| Total | 670 | 3,869 | 3,090 | 2,752 | 2,127 | 12,508 |

b. Number of samples submitted, by age and by region:

| | | | Samples Submited | | | |
| | | | Region | | | |
| Age | West | Mountain | Central | East | Multiregion | Total |
|---|---|---|---|---|---|---|
| 1-2 years | 28 | 161 | 178 | 413 | 109 | 889 |
| 3 years | 41 | 163 | 181 | 414 | 100 | 899 |
| 4 years | 100 | 410 | 430 | 1,053 | 178 | 2,171 |
| 5+ years | 492 | 3,107 | 2,293 | 832 | 1,723 | 8,447 |
| Unknown | 9 | 28 | 8 | 40 | 17 | 102 |
| Total | 670 | 3,869 | 3,090 | 2,752 | 2,127 | 12,508 |

c. Number of samples submitted, by month:

| Month | Samples Submitted |
|---|---|
| April 2002 | 809 |
| May 2002 | 1,492 |
| June 2002 | 1,234 |
| July 2002 | 990 |
| August 2002 | 1,158 |
| September 2002 | 1,179 |
| October 2002 | 1,325 |
| November 2002 | 926 |
| December 2002 | 828 |
| January 2003 | 825 |
| February 2003 | 832 |
| March 2003 | 910 |
| Total | 12,508 |

# Appendix II: U.S. Sheep Inventory and Operations

| | | NASS[1] Breeding Sheep 1-year and older January 1, 2003 | | | | Number of Operations with Sheep 2002 | |
| | | Ewes | Rams | Total | | | |
| Region | State | Head (1,000) | Head (1,000) | Head (1,000) | Pct. | Number (1,000) | Pct. |
|---|---|---|---|---|---|---|---|
| West | California | 295.0 | 10.0 | 305.0 | | 2,800 | |
| | Oregon | 125.0 | 7.0 | 132.0 | | 3,100 | |
| | Washington | 34.0 | 2.0 | 36.0 | | 1,200 | |
| | Total | 454.0 | 19.0 | 473.0 | 11.9 | 7,100 | 11.1 |
| Mountain | Arizona | 56.0 | 3.0 | 59.0 | | 270 | |
| | Colorado | 155.0 | 6.0 | 161.0 | | 1,900 | |
| | Idaho | 184.0 | 5.0 | 189.0 | | 1,000 | |
| | Montana | 213.0 | 8.0 | 221.0 | | 1,700 | |
| | Nevada | 65.0 | 2.0 | 67.0 | | 300 | |
| | New Mexico | 140.0 | 9.0 | 149.0 | | 800 | |
| | Oklahoma | 41.0 | 3.0 | 44.0 | | 1,500 | |
| | Texas | 680.0 | 40.0 | 720.0 | | 6,800 | |
| | Utah | 250.0 | 9.0 | 259.0 | | 1,400 | |
| | Wyoming | 295.0 | 10.0 | 305.0 | | 800 | |
| | Total | 2,079.0 | 95.0 | 2,174.0 | 54.6 | 16,470 | 25.7 |
| Central | Iowa | 135.0 | 7.0 | 142.0 | | 4,600 | |
| | Kansas | 47.0 | 2.5 | 49.5 | | 1,400 | |
| | Minnesota | 85.0 | 5.0 | 90.0 | | 2,300 | |
| | Missouri | 48.0 | 3.0 | 51.0 | | 1,600 | |
| | Nebraska | 56.0 | 3.0 | 59.0 | | 1,500 | |
| | North Dakota | 77.0 | 3.0 | 80.0 | | 1,000 | |
| | South Dakota | 265.0 | 10.0 | 275.0 | | 2,300 | |
| | Total | 713.0 | 33.5 | 746.5 | 18.8 | 14,700 | 22.9 |
| East | Illinois | 42.0 | 3.0 | 45.0 | | 2,200 | |
| | Indiana | 34.0 | 2.5 | 36.5 | | 2,000 | |
| | Michigan | 41.0 | 2.0 | 43.0 | | 1,900 | |
| | New England* | 32.0 | 3.0 | 35.0 | | 2,000 | |
| | New York | 40.0 | 3.0 | 43.0 | | 1,500 | |
| | Ohio | 97.0 | 6.0 | 103.0 | | 3,500 | |
| | Pennsylvania | 55.0 | 5.0 | 60.0 | | 2,600 | |
| | Virginia | 38.0 | 3.5 | 41.5 | | 1,500 | |
| | West Virginia | 23.0 | 1.0 | 24.0 | | 1,100 | |
| | Wisconsin | 52.0 | 3.0 | 55.0 | | 2,300 | |
| | Other States** | 89.0 | 12.0 | 101.0 | | 5,300 | |
| | Total | 543.0 | 44.0 | 587.0 | 14.7 | 25,900 | 40.3 |
| U.S. | | 3,789.0 | 191.5 | 3,980.5 | 100.0 | 64,170 | 100.0 |

1Source: National Agricultural Statistics Service (NASS), USDA; NASS Sheep and Goats, January 31, 2003
*New England includes CT, ME, MA, NH, RI, and VT
**Other States not published individually include AL, AK, AR, DE, FL, GA, HI, KY, LA, MD, MS, NJ, NC, SC, and TN. Note that AK and HI cannot be removed individually from this grouping.

# Appendix III: Scrapie: Ovine Slaughter Surveillance – Phase I Information Sheet

**APHIS**          **Info Sheet**

Veterinary Services
Centers for Epidemiology and Animal Health     **VS**        March 2003

## Scrapie: Ovine Slaughter Surveillance — Phase I

### Overview

The overall objective of the Scrapie: Ovine Slaughter Surveillance (SOSS) study is to estimate the national and regional prevalence of scrapie in mature cull sheep entering slaughter channels. The study was initiated at the request of members of the sheep industry, who wanted to accelerate eradication of the disease.

Beginning in February 2001 and ending in March 2002, Phase I included the study design, methods development, and sample-collection training. The objective of Phase I was to develop and modify the sample collection and testing processes, without emphasizing statistical results.

Phase II, which is the actual prevalence study, started April 1, 2002, and will continue for approximately 1 year. The number of samples to be collected from each participating plant on a weekly or bi-weekly basis was determined based on the number of cull ewes killed at the plant in 2001. Phase III will evaluate and analyze data received during Phase II. Phase III results will be published in fall 2003.

### Phase I Methods

The slaughter plants selected to participate in SOSS Phase I were identified based on historical slaughter data obtained through the Food Safety and Inspection Service and the National Agricultural Statistics Service. Since the currently accepted diagnostics are for adult animals, plants that slaughtered large numbers of mature cull ewes were targeted for the SOSS study.

Coordinators and sample collectors were selected for each State with a participating plant. Coordinators served as primary contacts in their States and were responsible for ensuring that the correct number of quality samples were submitted, supplies were ordered and provided to collection personnel, and information was disseminated as needed. Sample collectors were responsible for the actual collection of tissues at the slaughter plants.

Training the sample collectors was a critical component of SOSS Phase I and was accomplished via hands-on instruction and a Procedures Manual that identified the specific anatomical locations of the needed tissues. As the study progressed, regular field reports were produced to inform collectors of the quality of their submitted samples.

Established goals for submitting proper tissue in desirable condition were defined and met prior to Phase II of the study.

Diagnostic laboratories were contracted by the National Veterinary Services Laboratory (NVSL). Three contract labs were used for Phase I. Before SOSS tissues were submitted, laboratory personnel were trained and the labs were approved by NVSL.

To remain consistent with Phase I objectives, convenience sampling, which is sampling at will and without regard to randomness, was used for all samples taken during Phase I.

A standard kit was used that included tools and materials to collect, package, and ship the needed tissues to NVSL.

Samples were collected from the brainstem (obex), cerebellum, both tonsils, both retropharyngeal lymph nodes, and the lymphoid tissue of the third eyelid.

At the time of tissue collection, data regarding each head were recorded on a submission form. These data included a unique sample number, animal identification, face color, and age of the animal based on the number and condition of permanent teeth. Ear tags and plant or market records (when available), and the Veterinary Services generic data base were used to determine the region of animal origin.

### Phase I Findings

SOSS Phase I was a "pilot" project. Samples were not collected with the intention of performing rigorous statistical analysis. Therefore, results shown below do not reflect the U.S. cull ewe population.

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USDA APHIS VS / 27

Tissue samples were collected from 1,159 cull ewes in 23 slaughter plants from 13 States.

| Sheep Face Color | Number | Percent |
|---|---|---|
| White | 648 | 55.9 |
| Black | 318 | 27.4 |
| Mottled | 143 | 12.4 |
| Not recorded | 50 | 4.3 |
| Total | 1,159 | 100.0 |

| Age of Sheep | Number | Percent |
|---|---|---|
| 1 year | 54 | 4.7 |
| 2 years | 127 | 10.9 |
| 3 years | 201 | 17.3 |
| 4 years | 407 | 35.1 |
| 5 years or older | 338 | 29.2 |
| Not recorded | 32 | 2.8 |
| Total | 1,159 | 100.0 |

Tissues were tested using the immunohistochemistry technique (IHC).

| Tissue Test Result (IHC) | Obex | Tonsil | Lymph Node |
|---|---|---|---|
| Negative | 1,079 | 939 | 1,139 |
| Positive | 2 | 2 | 2 |
| Untestable | 78 | 218 | 18 |
| Total | 1,159 | 1,159 | 1,159 |

For more information, contact:

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#N390.0303

Genotype results were obtained for 611 of the 1,159 samples.

| Genotype Results | Number | Percent |
|---|---|---|
| QQ | 237 | 20.4 |
| QR | 279 | 24.1 |
| RR | 95 | 8.2 |
| XX (not testable) | 44 | 3.8 |
| Not tested | 504 | 43.5 |
| Total | 1,159 | 100.0 |

Two animals out of the 1,159 tested were positive for scrapie. In these animals, all three tissues (obex, tonsil, and lymph node) tested positive. Both were 4-year-old ewes and were QQ genotypes. One was white -faced, the other black-faced. Neither sample could be identified back to the region of origin.

**Lessons Learned**

Phase I allowed for the development of plant relations, modification of forms, and the fine-tuning of tissue collection protocols before the implementation of the full study in April of 2002.

Since approximately two-thirds of all mature cull ewes are exported to Mexico annually, a livestock market sampling protocol was developed for Phase II that calls for the sampling of sheep that are typically exported to Mexico and not sent to domestic slaughter plants.

An instructional CD-ROM containing video clips, color pictures, and the SOSS instruction manual was developed and distributed for Phase II. By the time Phase II began, proficiency (percent testable tissues) for each tissue type was at least 80 percent.

Collecting third eyelid tissue from dead animals was extremely difficult and was stopped a few months into Phase I.

Additional diagnostic laboratories were contracted by NVSL to assist with the increased workload during Phase II.

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# Appendix IV: Laboratory Selection and Results

a. Participating laboratories

**Immunohistochemistry:**

University of California – Davis (CAHFS)

Colorado State Universtiy Diagnostic Lab

Cornell University Animal Health Diagnostic Laboratory

Kansas State University

Michigan State University

National Veterinary Services Laboratory (NVSL)

University of Nebraska Veterinary Diagnostic Center

Ohio Department of Agriculture Animal Disease Diagnostic Laboratory

Texas Veterinary Medical Diagnostic Laboratory

Wyoming State Veterinary Laboratory

**Genetics:**

USDA Agricultural Research Service – Pullman, Washington

University of California – Davis

GeneSeek

Qiagen

b. Sample distribution and positive results:

| Laboratory | Samples | | | |
| --- | --- | --- | --- | --- |
| | Number Heads Tested | Percent of Total Tested | Number Positive | Percent of Total Positives |
| University of California – Davis | 2,377 | 19.0 | 6 | 18.2 |
| Colorado State University Diagnostic Lab | 1,799 | 14.4 | 8 | 24.3 |
| Cornell University Animal Health Diagnostic Laboratory | 241 | 1.9 | 2 | 6.1 |
| Kansas State University | 284 | 2.3 | 1 | 3.0 |
| Michigan State University | 968 | 7.7 | 3 | 9.1 |
| National Veterinary Services Laboratory | 1,064 | 8.5 | 1 | 3.0 |
| University of Nebraska Veterinary Diagnostic Center | 370 | 3.0 | 1 | 3.0 |
| Ohio Department of Agriculture Animal Disease Diagnostic Laboratory | 572 | 4.6 | 1 | 3.0 |
| Texas Veterinary Medical Diagnostic Laboratory[1] | 2,232 | 17.9 | 4 | 12.1 |
| Texas Veterinary Medical Diagnostic Laboratory[2] | 1,256 | 10.1 | 2 | 6.1 |
| Wyoming State Veterinary Laboratory | 1,328 | 10.6 | 4 | 12.1 |
| Total | 12,491 | 100.0 | 33 | 100.0 |

[1]Amarillo location; [2]Texas-College Station location

# Appendix V: Age Determination by Teeth Examination
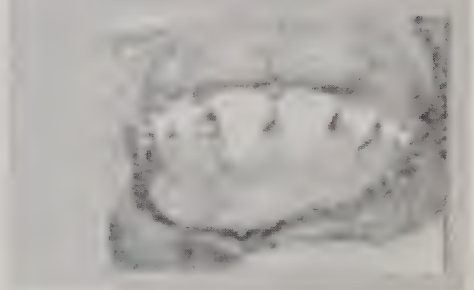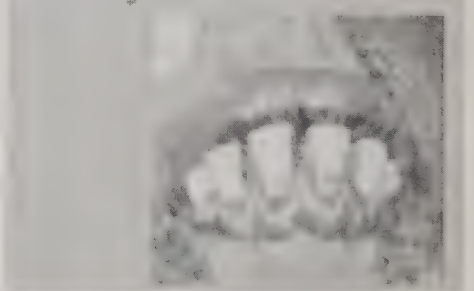

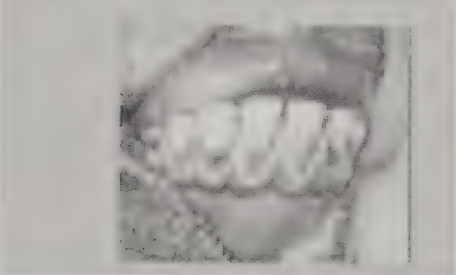Observe size of first incisors.


Observe size of first incisors.


Observe first incisors and eruption
of second incisors.


Observe eruption of third incisors.


Adult sheep with full mouth.
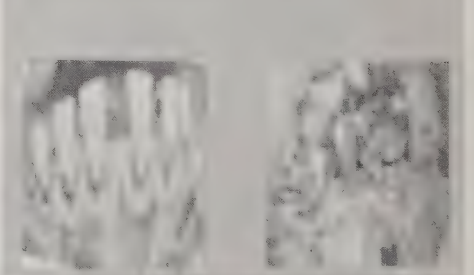

Broken mouth (sheep 5 years and
older, incisors erupted).